

Research article

Micrographic Profiling and Phytochemical Analysis of Some Plants Consumed by *Okapia johnstoni* (Giraffidae: Mammalia) in Democratic Republic of the Congo

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Abstract: The aim of this study was to determine the phytochemical composition and micrographic characteristics of the plants consumed by *Okapia johnstoni*. The results indicate that each plant species has characteristic microscopic elements for its identification. These plants are rich in phenolic acids, anthocyanins, flavonoids, anthraquinones, coumarins, terpenoids and iridoids. *Alchornea cordifolia* is richer in total polyphenols (198.53±3.39 mg GAE/g DM) followed respectively by *Musanga cercopioides* (91.87±6.71 mg GAE/g DM), *Macaranga spinosa* (59.65±6.54 mg GAE/g DM), *Ficus vallis-choudae* (46.37±2.43 mg GAE/g DM), *Cola acuminata* (38.83±4.04 mg GAE/g DM), *Pycnanthus angolensis* (31.96±3.45 mg GAE/g DM), *Alstonia boonei* (31.55±1.60 mg GAE/g DM) and *Trilepisium madagascariensis* (25.18±0.99 mg GAE/g DM). As for flavonoids, the highest content is obtained in *T. madagascariensis* followed respectively by *A. boonei*, *Pycnanthus angolensis*, *Cola acuminata*, *M. spinosa*, *F. vallis-choudae*, *M. cercopioides* and *A. cordifolia*. The difference in the content of secondary metabolites is justified by the fact that their expression in the plant is a function of both abiotic and biotic factors and the specificity of each plant species linked to its genetic make-up. The characterization of these chemical compounds is necessary for the formulation of herbal medicines for the management of Okapi *ex situ* or for human health. Also, the microscopic profiles of the leaves powder of the studied plant species provide relevant information, which may be helpful for the plant authentication and for quality control of raw material.

Keywords: Okapi (*Okapia johnstoni*), self-medication, conservation, Abumombazi Forest Reserve, Ubangi eco-region

1. Introduction

The Okapi (*Okapia johnstoni* Sclat) is a species of mammalian ruminant belonging to the family of Giraffidae (Order Artiodactyla: Mammalia) with solitary and discreet

behaviour [1]. This endemic animal of the Democratic Republic of the Congo (DRC) feeds mainly on leaves, buds, tender branches, fruits, mushrooms, and ferns, but also on clay as a source of minerals (Figure 1). According to international biodiversity conventions, Okapi is classified on the threatened species red list [2, 3]. Recent confirmation of its presence in North Ubangi Province (DRC) by the team of Professor Jean-Paul Ngbolua Koto-Te-Nyiwa of the University of Kinshasa (in collaboration with the University of Gbadolite, North Ubangi) has reignited renewed interest both nationally and internationally in conserving this emblematic animal in other politically stable regions of the country that meet the criteria for high conservation value sites [4]. The present study was initiated as part of the project to create an Okapi urban park in Gbadolite city, North Ubangi Province, for the purpose of conserving this emblematic and endemic animal. It has recently been postulated that the choice of certain forages over others is a determining factor in the survival of Okapi both in their natural environment and in captivity, concerning their behavior and reproductive abilities. Thus, it could be a matter of self-medication (zoo pharmacognosy) as the death of these animals is usually attributed to intestinal and infectious diseases [5-7]. Considering that the need to respond to such pressure is great due to co-evolution, we can expect that Okapi historically developed self-medicating behaviors by resorting to chemical defenses of plants to protect themselves from parasites, as is the case in non-human primates [8]. In this research, the hypothesis is that the plant species consumed by *Okapia johnstoni* contain phytochemicals that act either individually or synergistically to increase their chances of survival in a hostile environment such as a tropical forest. The present study aims to determine the qualitative and quantitative phytochemical composition of secondary metabolites of plants consumed by *Okapia johnstoni*.

The relevance of the present work is obvious because it allows the creation of a database on the phytochemistry of Okapi forage plants, knowledge necessary for the formulation of herbal medicines based on these plants to treat Okapi *ex situ*.

2. Materials and Methods

2.1. Plant material and handling

Samples used in this study were leaves of *Alchornea cordifolia*, *Alstonia boonei*, *Cola acuminata*, *Ficus vallis-choudae*, *Musanga cecropioides*, *Macaranga spinosa*, *Pycnanthus angolensis*, and *Trilepisium madagascariensis*. The identity of the plants was established by Mr. Justin A. Asimonyio, botanist and researcher at the Biodiversity Monitoring Center (Faculty of Science, University of Kisangani, Democratic Republic of the Congo). Samples were air-dried at room temperature. Prior to extraction, they were ground and stored in brown covered glass bottles.

2.2. Optical micrography assessment of powders

Powder observations were made using lactic acid reagent (European Pharmacopeia reagent)[11]. For the microscopic analysis, two drops of lactic acid reagent dropped on the slide were mixed with a small quantity of powder and then covered with a cover glass. The obtained microscopic preparation was warmed up to boiling [9]. Observations and pictures were made with OLYMPUS Model CH10BIMF microscope and pictures were taken with Smart Phone Samsung S9.

2.3. Phytochemical profiling using thin layer chromatography (TLC)

Two different types of total extracts were prepared. They are methanol extract (100 mg/mL: research of flavonoids, phenolic acids, iridoids, anthocyanins, anthraquinones, and coumarins) and acetate extract (100 mg/mL: research of terpenoids). For flavonoids and phenolic acid profiling, the system used is the mixture of Ethyl acetate/formic acid/acetic acid/water (100: 11:11: 27) and the detection reagent used is Neu's reagent.

The system based on the mixture of Ethyl Acetate/Methanol/Water (100: 13.5 : 10) and the detector consisting of sulfuric alcohol were used for the detection of iridoids, while the system of Toluene/Ethyl Acetate (9 : 1 v/v) as well as the revealing Anisaldehyde sulfuric reagent were used for the detection of terpenoids. The ethyl acetate/formic acid/water system (100: 10: 40 v/v) and the Vanillin phosphoric, as well as the ethyl acetate/methanol/water system (100: 13.5: 10 v/v) and the KOH alcoholic were used for the detection of anthocyanins and anthraquinones. On the other hand, the Toluene/Ether system (1: 1 v/v) and alcoholic KOH reagent were used to highlight coumarins [9].

2.4. Estimation of *Phytomarkers content*

Samples for quantitative analyses were prepared from 10 mg of each extract dissolved in 50 ml of solvent (methanol).

2.4.1. Total polyphenols

- **Preparation of the Folin-Ciocalteu reagent**

Ten grams (10 g) of sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) and 2.5 g of sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) are dissolved in 70 ml of distilled water. Add 50 ml of 85% phosphoric acid (H_3PO_4) ($d=1.71$) and 10 ml of 36% concentrated hydrochloric acid ($d=1.19$). Boil under reflux for 10 hours, then add 15 g of lithium sulfate (Li_2SO_4), some drops of bromine, and boil once again for 15 minutes. Cool and adjust to 100 ml with distilled water.

- **Total polyphenols content estimation**

Total available polyphenol content was determined by the Folin-Calciolteu method. A quantity of 200 microliters of the extract is mixed with 1ml of freshly prepared Folin-Calciolteu reagent (10 times diluted) and 0.8ml of 7.5% sodium carbonate (Na_2CO_3). The whole is incubated at 50 °C for 30 minutes and reading is done against a blank using a spectrometer at 765 nm. Total polyphenol content (expressed as gallic acid equivalent) is given by the relation: $Y = 0,006x - 0,002$; $R^2 = 0,997$.

- **Flavonoids**

The flavonoid content of the extracts was determined using the aluminum trichloride colorimetric method. A quantity of 100 microliters of the extract was mixed with 4 ml of distilled water and then 0.3 ml of a 5% sodium nitrite (NaNO_2) solution. After some minutes, 0.02 ml of a 10% AlCl_3 solution was added. 2 ml of 1M Na_2CO_3 solution was added to the mixture and the whole was diluted with 10 ml of distilled water after 5 minutes break. The absorbance was measured at 510 nm. The flavonoid content (expressed as quercetin equivalent) is given by the relation: $Y = 0.009x + 0.006$; $R^2 = 0.999$.

3. Results and Discussion

3.1. *Okapi and Microscopic profile*

The results of the optical micrography of powders plant species consumed by *Okapia johnstoni* (Figure 1) revealed the presence of several histological characteristic elements as show in Figures 2-9.



Figure 1. *Okapia johnsoni*

The typical elements of *A. Cordifoliola* leaves were diacytic stomata (A), crystal fiber fragments (B), elongated palisade cells (C) and epidermal cell fragments (D) (Figure 2). In the *Alstonia boonei* leaves, it was observed evidence of hexagonal isodiametric epidermal cells (A), spiral vessel fragments (B), unicellular trichomes with a finely granular surface (C), and elongated fiber fragments (D) (Figure 3). *Ficus vallis choudae* leaves showed the presence of smooth unicellular non-glandular trichomes (A), punctate vessel fragments (B), parenchyma fragments (C), and palisading cell fragments (D) (Figure 4). Powder microscopy of *Musanga cercopiooides* leaves, revealed the presence of crystalline fibers (A), fragments of hexagonal epidermal cells (B), numerous unicellular branched or arborescent trichomes (C) and fragments of spiral vessels (D) (Figure 5). *Macaranga spinosa* leaf powder showed in the same manner the presence of sea urchin calcium crystals (A), long smooth unicellular non-glandular trichomes (B), spiral vessels (C) and fragments of palisading cells (D) and also polygonal epidermal cells (Figure 6). The powder from leaves of *Pycnathus angolensis* demonstrated the presence of punctate vessel fragments (A), suber fragments (B), epidermal cells (C) and fiber fragments (D) and several distinct stomata (Figure 7). In *Cola acuminata* leaf powder, we noticed the presence of diacytic stomata (A) and fragments of spiral vessels (B) (Figure 8). The examination of *Trilepisium madagariensis* leaf powder revealed the presence of paracytic stomata (A), lower epidermal cells (B), upper epidermal cells (C) and fiber fragments (D) (Figure 9).

Histological elements obtained for each species are hence characteristic of the leaf powders of the plants studied. According to the best of our knowledge, there is no data on the microcopy of the powders of these plants. These characteristic elements constitute a database for further studies.

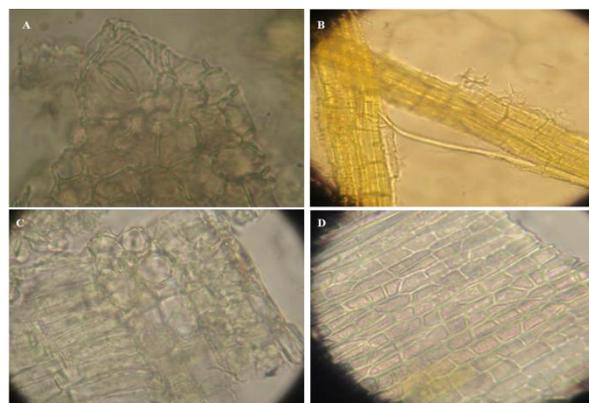


Figure 2. Micrographic features of *A. Cordifoliola*: stomata (A), crystal fiber fragments (B), elongated palisade cells (C) and epidermal cell fragments (D).

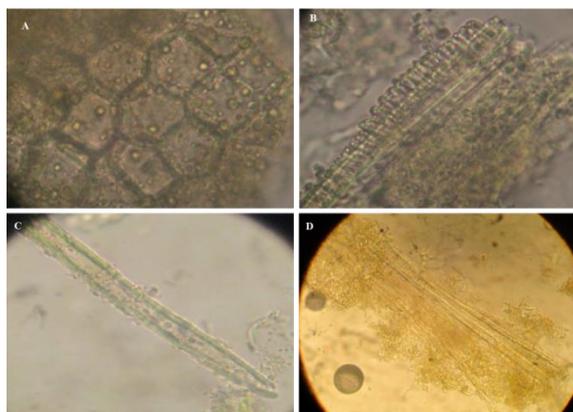


Figure 3. Microscopic features of *A. boonei*: hexagonal isodiametric epidermal cells (A), spiral vessel fragments (B), unicellular non-glandular trichomes with a finely granular surface (C), and elongated fiber fragments (D).

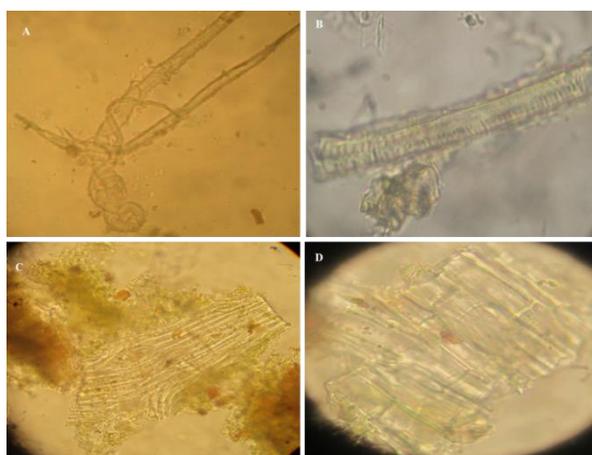


Figure 4. Microscopic features of *Ficus vallis-choudae*: smooth unicellular trichomes (A), punctate vessel fragments (B), parenchyma fragments (C), and palisade cell fragments (D).

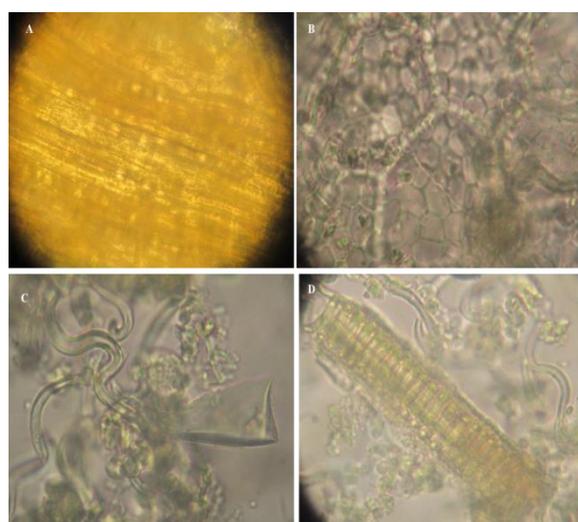


Figure 5. Microscopic properties of *Musanga cercopioides*: crystalline fibers (A), fragments of hexagonal epidermal cells (B), numerous unicellular branched or arborescent trichomes (C) and fragments of spiral vessels (D).

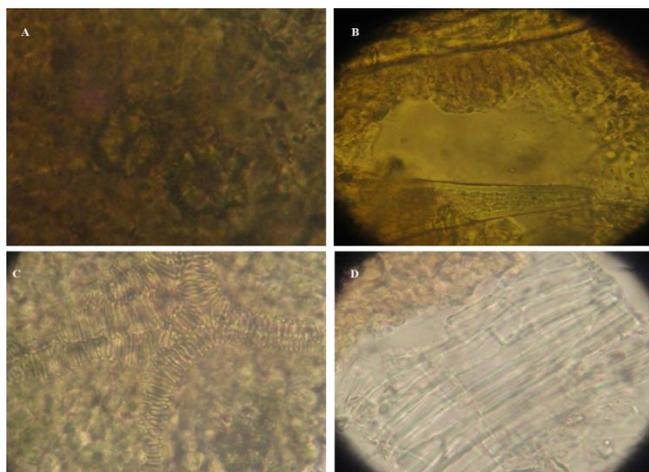


Figure 6. Microscopic profile of *Macaranga spinose*: oxalate calcium crystals (A), long smooth unicellular trichomes (B), spiral vessels (C) and fragments of palisade cells (D) and also polygonal epidermal cells.

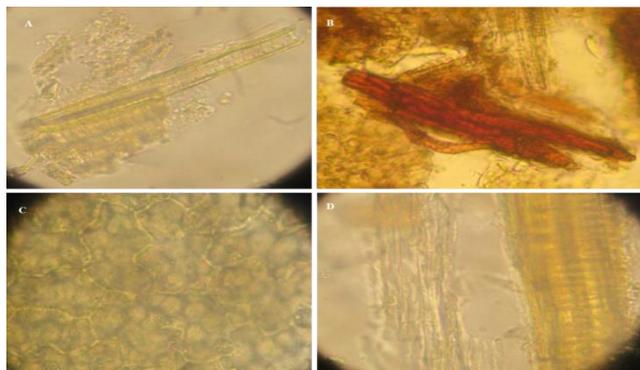


Figure 7. Microscopic profile of *Pycnathus angolensis*: punctate vessel fragments (A), suber fragments (B), epidermal cells (C) and fiber fragments (D) and several distinct stomata.

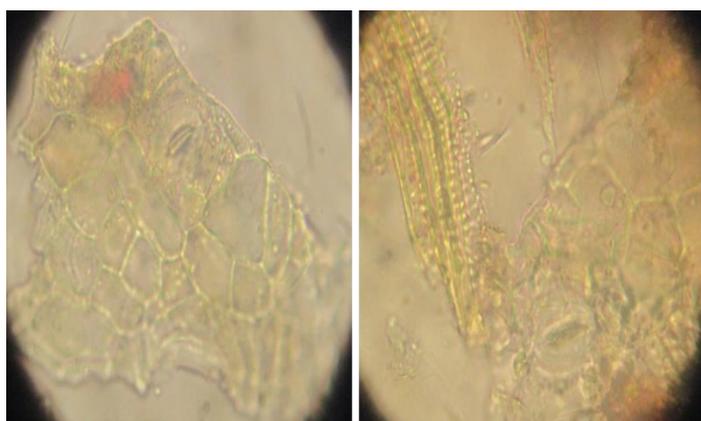


Figure 8. Microscopic features of *Cola acuminata*: diacytic stomata (A) and fragments of spiral vessels (B).

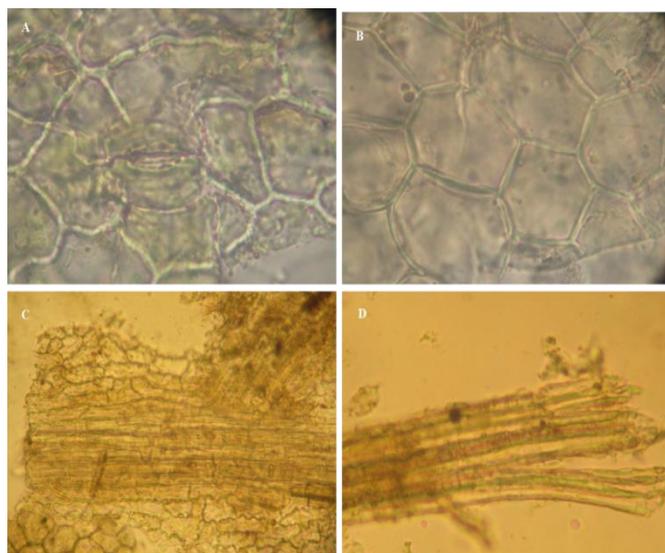


Figure 9. Microscopic characteristics of *Trilepisium madagariensis*: paracytic stomata (A), lower epidermal cells (B), upper epidermal cells (C) and fiber fragments (D).

The microscopic profiles of the leaves powder of the studied plant species provide relevant information, which may be helpful for the plant authentication and for quality control of raw material.

3.2. Phytochemicals

Figures 10-15 give the TLC profiles of studied plant species.

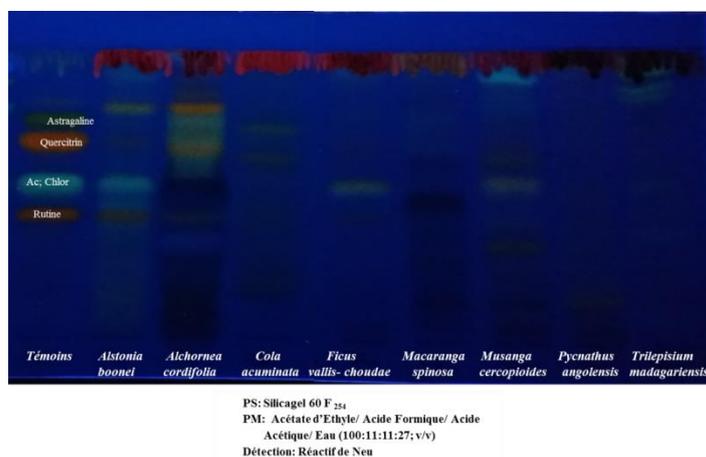


Figure 10. TLC chromatogram of the methanolic extracts of the plants at 366 nm [System: Ethyl acetate/formic acid/acetic acid/water (100: 11:11: 27), Detection: Neu reagent].

Figure 10 shows the presence of flavonoids (yellow, orange, orange-yellow and green fluorescent spots) and phenolic acids (blue fluorescent spots) in all extracts. The extracts richest in flavonoids are those of *A. boonei*, *A. cordifolia*, *C. acuminata* and *M. cercopitoides*. In the presence of standards (or controls), *A. boonei* would contain chlorogenic acid and rutin; *A. cordifolia* would also contain rutin. *M. cercopitoides* would contain caffeic acid, which corresponds to the intense blue fluorescent spot on the chromatographic profile. Each extract showed a characteristic chromatographic profile. Flavonoids are widely distributed compounds in the plant kingdom. Research indicates that flavonoids are involved in the prevention of cancer; indeed, added to the diets of various laboratory animals, they limit the development of tumors induced experimentally by exposure to carcinogenic agents. They

are efficient against many cancers (colon, stomach, liver, breast, prostate, lung, skin, bladder, etc.). They are reported to have various activities, such as antiviral, anti-tumor, anti-inflammatory, anti-allergic, anti-cancer, anti-ulcer, anti-carcinogenic, antimicrobial, etc. properties [10]. Thus, these secondary metabolites may play a preventive and/or curative role in the health of okapi *ex situ*.

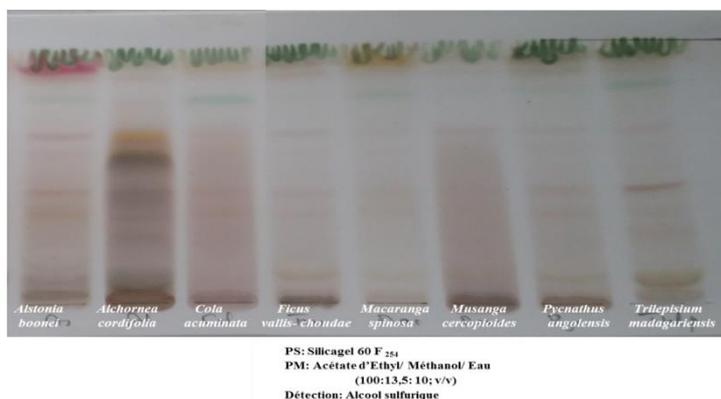


Figure 11. TLC chromatogram of the methanol extracts of the plants studied in the visible [System: Ethyl acetate/Methanol/Water (100: 13.5: 10), Detection: sulfuric alcohol].

The analysis of the chromatogram in Figure 11 provides information on the presence of iridoids (colored spots) in the different extracts whose nature remains to be determined. The plants that were richer in iridoids are *A. boonei*, *A. cordifolia* and *C. acuminata*. The black spots correspond to the terpenoids. Iridoids exhibit a wide range of biological activities due to their structural features. They possess antimicrobial [11], neuro-protective, hepato-protective, anti-tumor antioxidant, anti-inflammatory, hypoglycemic and hypolipidemic properties [12]. Therefore, the presence of these secondary metabolites in the leaves of these different plants analyzed can be very useful for the health of Okapi *ex situ*.

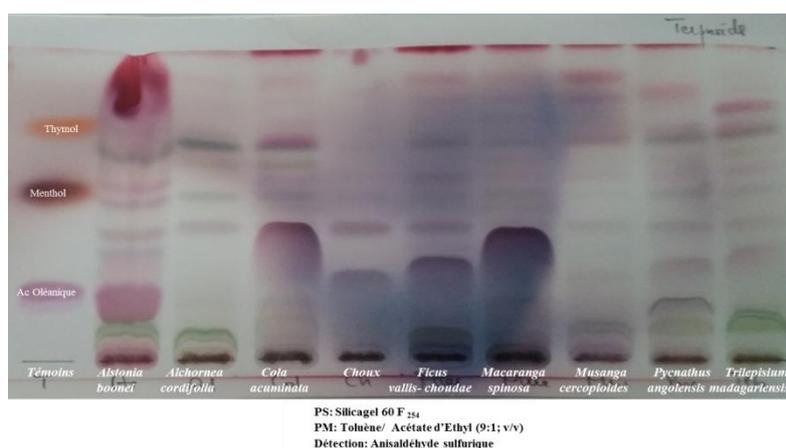


Figure 12. TLC chromatogram of ethyl acetate extracts of the plants studied in the visible [System: Toluene/Ethyl acetate (9: 1 v/v), Detection: Sulfuric anisaldehyde]

Figure 12 provides information on the richness of the different plants in various terpenoids revealed by the presence of purplish, red-orange spots. The different terpenoids remain to be characterized. Compared to the controls, none of the plants contained menthol, thymol and oleanolic acid. Terpenoids are known to have antifungal, antibacterial, anti-inflammatory, anti-tumor, antibacterial, antiviral, antimalarial, antidiabetic, and other properties [13]. The presence of these secondary metabolites in these different plants consumed by *Okapia johnsoni* may positively impact its life in captivity. Among the plants

studied, *A. cordifolia*, *C. acuminata*, *F. vallis-choudae* and *M. cercopoides* were found to be rich in anthocyanins as shown in the chromatogram above (Figure 13).

Experimental studies indicate that anthocyanins are free radical scavengers and are considered inhibitors of cancer cell proliferation [14]. Their antioxidant activity suggests that their dietary intake could play a beneficial role in human health, particularly in the prevention of cardiovascular disease. Anthocyanins are also known for their anti-sickling activity [15-19]. Thus, the presence of these chemical groups may be useful for the health of *Okapia johnsoni* in the prevention and therapy of diseases caused by free radicals outside its native environment.

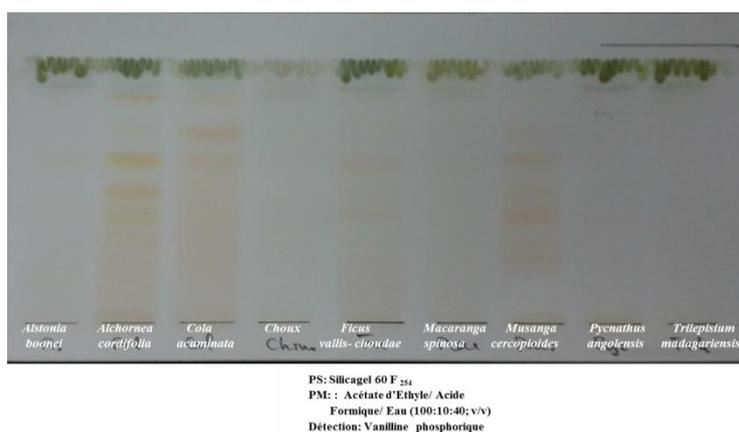


Figure 13. TLC chromatogram of the methanolic extracts of the plants studied in the visible [System (Ethyl acetate/formic acid/water (100: 10: 40 v/v), Detection: phosphoric vanillin)]

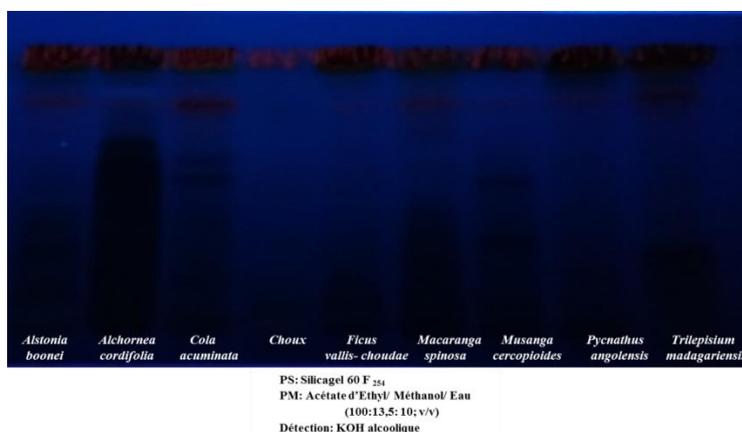


Figure 14. TLC chromatogram of methanolic extracts of the studied plants at 366 nm [System (Ethyl acetate/Methanol/Water (100: 13.3: 10 v/v), Detection: alcoholic KOH)]

Figure 14 demonstrates that after revelation with alcoholic KOH, the red fluorescent spots thus indicating the presence of anthraquinones. These compounds are present in *A. boonei*, *C. accuminata*, *M. spinosa*, *P. angolensis* and *T. madagariensis*. On the other hand, they are absent in *M. cercopoides*, *A. cordifolia*, and *F. vallis-choudae*. In fact, anthraquinones are an important class of compounds that have been used for centuries in medical treatments. They have antioxidant, anti-tumor, antimicrobial, anti-parasitic, estrogenic, topoisomerase inhibitory and antidiabetic properties [20, 21]. Regarding coumarins, they were detected in all plants studied. Coumarins are represented by blue fluorescent spots at 366 nm (Figure 15). Coumarins are biologically active molecules exhibiting important antimicrobial, anti-inflammatory, anti-tumor, anti-hypertensive, antioxidant, cytochrome P-450 inhibitory and neuroprotective activities [22].

The overall results of the qualitative chemical composition show that all these plants consumed by Okapi are rich in secondary metabolites endowed with remarkable pharmacological properties. The different plants studied could be useful and would play a preventive and/or curative role for the health of this animal in captivity.

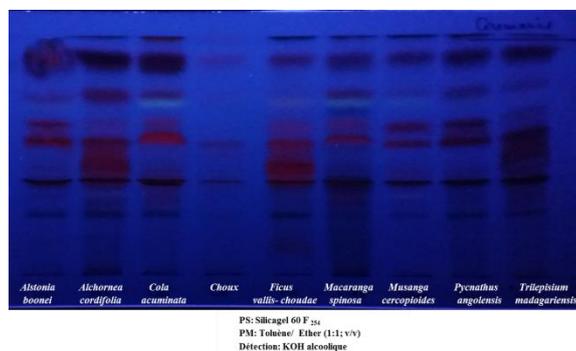


Figure 15. TLC chromatogram of the methanol extracts of the studied plants [System (Toluene/Ether (1: 1 v/v), Detection: alcoholic KOH)]

The [Table 1](#) gives the results of total polyphenol and flavonoid content of different Okapi food plant species.

Table 1. Total polyphenol and flavonoid content of different Okapi food plants

Plant species	Total polyphenols (mg GAE/g MS)	Flavonoids (mgEQ/g DM) (R)
<i>A. boonei</i>	31,55±1,60	30,53±0,07 (0,8613)
<i>A. cordifolia</i>	198,53±3,39	58,79±1,46 (0,2965)
<i>C. acuminata</i>	38,83±4,04	26,68±1,08 (0,6870)
<i>F. vallis-choudae</i>	46,37±2,43	25,42±2,18 (0,5481)
<i>M. spinosa</i>	59,65±6,54	33,35±0,31 (0,5590)
<i>M. cercopioides</i>	91,87±6,71	37,31±0,18 (0,4061)
<i>P. angolensis</i>	31,96±3,45	24,46±0,85 (0,7630)
<i>T. madagascariensis</i>	25,18±0,99	21,69±1,20 (0,9676)

Legend: (mg GAE/g DM) mg Gallic acid equivalent per g dry matter; (mgEQ/g DM) mg Quercetin equivalent per g dry matter; R=flavonoids/total polyphenols ratio

From [Table 1](#), it is evident that *A. cordifolia* is most rich in total polyphenols followed respectively by *M. cercopioides*, *M. spinosa*, *F. vallis-choudae*, *C. acuminata*, *P. angolensis*, *A. boonei* and *T. madagascariensis*. As for flavonoids, the highest content is obtained in *T. madagascariensis* followed respectively by *A. boonei*, *P. angolensis*, *C. acuminata*, *M. spinosa*, *F. vallis-choudae*, *M. cercopioides* and *A. cordifolia*. The difference in the concentration of these secondary metabolites is justified by the fact that their expression in the plant is a function of both abiotic factors such as climate, geological environment of the plant harvesting site, etc. and biotic factors such as the presence of predators and/or parasites as well as inter-specific competitions between plants within ecosystems and the specificity of each plant species related to its genetic makeup [19]. Furthermore, in a recently published study [2], it was shown that these plants contain Calcium, Iron, Magnesium, cyanogen compounds fat and crude proteins. The content of these plants consumed by Okapi would be similar to that of *Cynodon dactylon*, the plant species widely consumed by cattle. These same studies also showed that *Cola acuminata*, which is the plant less consumed than *Alchornea cordifolia*, is nevertheless more energetic than the latter plant. This permitted us to justify in

part the self-medication behavior of the Okapi. In this regard, *Alchornea cordifolia*, *Alstonia boonei*, and *Ficus vallis-choudae*, which are less energetic, would be consumed because of their medicinal properties (presence of secondary metabolites such as total polyphenols and flavonoids). Indeed, according to [23], all these plants are known in traditional African medicine as having medicinal properties. The secondary metabolites detected in these plants have also been found in other plants with antihelminthic properties and could be used for treating Okapi in *ex situ* conservation [24, 19].

In Africa, it is well known that it is only in the DRC that the Okapi is found, especially in the East of the country and, in particular, at the Epulu Okapi Wildlife Reserve (RFO). However, it should be noted that this emblematic animal is also found in the North-Ubangi province [4]. However, in the east of the country, its habitat is increasingly fragmented due to anthropogenic pressure. In addition, the RFO is a victim of poaching and recurrent armed conflicts. Hence, the interest in protecting this animal species in other geographical areas of the Republic where this animal is endemic, including the forest reserve of Abumombazi, which meets the criteria for sites of high conservation value.

Additionally, the ethno-botanical survey reported that almost these plants are also used in various human pharmacopeia to treat illnesses. For example, *Cola acuminata* is widely used for treating bacterial infections [25]. *Alstonia boonei* is employed against chronic diarrhea dysentery, fever, pain, intestinal disorder, bacterial infections and diabetes [26-28]. *Ficus vallis choudae* is used against poisons, giddiness, jaundice, haemorrhoids and epilepsy [29]. *Alchornea cordifolia* is known to treat several diseases such as rheumatism, arthritis, colds, muscle pains, cough, infertility, impotence, diabetes, diarrhea, gonorrhoea, anaemia, dermatitis, malaria, dysentery, toothache, Stomach pains, tooth aches, urinary, respiratory and gastro-intestinal disorders, asthma, and skin infections [30-32]. *Musanga cecropioides* is recognized as a good remedy against arterial hypertension, constipation, pain during childbirth, cough, diabetes, schizophrenia, fever, jaundice, acute gastric poisoning, liver diseases, helminths and dysenteric [33,34]. *Pycnanthus angolensis* help to cure various pains, helminths, inflammatory, pneumonial infections, stomach pain, chest pain, rhinitis problems, malaria, toothache, fungal skin infections, worms and leprosy [35,36]. Hence, these plants are useful both for human and animal health.

5. Conclusion and Suggestions

The aim of this study was to assess the qualitative and quantitative phytochemical composition and micrographic characteristics of plants consumed by *Okapia johnstoni*. From this study, it was found that the leaves of each plant species have characteristic microscopic elements for their identification. All plants tested are rich in various secondary metabolites such as phenolic acids, anthocyanins, flavonoids, anthraquinones, coumarins, terpenoids, and iridoids. The secondary metabolites detected in these plants are also known to have antihelminthic properties and could be used to treat Okapi in *ex situ* conservation in addition to the energetic value of these phyto-resources. It is therefore desirable to protect the Okapi in other geographical areas of DRC where this animal is endemic, in particular the Abumombazi forest reserve, which meets the criteria for sites of high conservation value. Phytochemical studies for the isolation and characterization of chemical compounds with antihelminthic properties are necessary in order to formulate herbal medicines for the management of these animals *ex situ* or for human health. Also, the microscopic profiles of the leaves powder of the studied plant species provide relevant information, which may be helpful for plant authentication and for quality control of raw material.

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